EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
ASSURANCE SARS-COV-2 PANEL
(Assurance Scientific Laboratories)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

INTENDED USE

The Assurance SARS-CoV-2 Panel is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasal, nasopharyngeal or oropharyngeal swabs, from individuals suspected of COVID-19 by their healthcare provider.

This test is also for use with nasal swab specimens that are self-collected at home or in a healthcare setting by individuals using an authorized home-collection kit when determined to be appropriate by a healthcare provider

Testing is limited to Assurance Scientific Laboratories (Birmingham, AL) that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263, to perform high-complexity tests.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Assurance SARS-CoV-2 Panel is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures. The Assurance SARS-CoV-2 Panel test is only for use under the Food and Drug Administration's Emergency Use Authorization.

2) Special Conditions for Use Statements

For Emergency Use Authorization (EUA) only
For prescription use only
For *in vitro* diagnostic use

DEVICE DESCRIPTION AND TEST PRINCIPLE

The assay is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines.

Sample Preparation

Two extraction chemistries are validated for COVID-19 PCR testing: Abnova Precipitor32 or Indical Indimag 48 (using the Zymo Quick-RNA Viral Kit RNA Extraction Kit). The underlying workflow involves adding a lysis buffer that will disrupt cellular material and release nucleic acids. The lysis buffer inactivates nucleases present in the specimen. Magnetic silica is added to the lysed specimen and under high salt concentrations, the nucleic acids bind to the magnetic silica. Following two washes, the nucleic acids are eluted from the magnetic silica into the elution buffer.

Amplification

Detection of SARS-CoV-2 RNA uses reverse transcriptase PCR (RT-PCR) to detect the viral envelope (E) gene. This portion of the genome is conserved in other bat-derived betacoronaviruses and not conserved among other coronaviruses. RT-PCR amplifies RNA targets by first producing cDNA from the RNA target. The cDNA is then amplified by PCR. The TaqPath 1-Step RT-qPCR Master Mix allows this process to proceed without the addition of reagents between the RT and PCR steps.

The addition of a TaqMan probe serves to eliminate detection of nonspecific amplification in the reaction. The probe consists of an oligonucleotide with a 5'-reporter dye (FAM) and a 3'-quencher dye (BBQ). If the target is present, the probe will anneal between the forward and reverse primer sites. In this setting, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. The 3' end of the probe is blocked so that the probe cannot be extended during PCR. DNA polymerase exonuclease activity cleaves the TaqMan probe during PCR. This separates the reporter dye from the quencher dye, resulting in increased fluorescence of the reporter. This allows detection of the accumulation of PCR products.

Detection

The BioRad CFX96 or CFX384 is used for qualitative and quantitative detection with fluorescent-based PCR chemistries. During PCR, light from a lamp is focused on each well of the microplate. The light excites the fluorescent dye in each well and emission between 500 nm and 600 nm is detected. The system allows data analysis and reporting in a variety of formats.

INSTRUMENTS USED WITH TEST

Instruments

The Assurance Scientific Laboratories SARS-CoV-2 real-time RT-PCR test is to be used with the Abnova Precipitor32 or Indical Indimag 48 (using the Zymo Quick-RNA Viral Kit RNA Extraction Kit) and the BioRad CFX96 and BioRad CFX384 with the BioRad CFX Maestro software.

Collection Kits

This assay can be used with the Everlywell COVID-19 test home collection kit. Everlywell has granted Assurance Scientific Laboratories a right of reference to the data supporting the use of this authorized home collection kit.

Reagents

The primary reagents used in this assay, including primer and probe designs, are adapted from the "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel" document effective March 30, 2020.

Kits and Reagents	Manufacturer	Catalog #
Abova Precipitor32 Abova Precipitor32: Viral Total Nucleic Acid Purification Kit	Abnova	U0382
Zymo Quick-RNA Viral Kit RNA Extraction Kit	Zymo	R2140 or R2141
TaqPath 1-Step RT-qPCR Master Mix, CG	ThermoFisher	A15299
Primer: COVID-19_N1-F	IDT or Biosearch	Custom
Primer: COVID-19_N1-R	IDT or Biosearch	Custom
Probe: COVID-19_N1-P	IDT or Biosearch	Custom
Primer: COVID-19_N2-F	IDT or Biosearch	Custom
Primer: COVID-19_N2-R	IDT or Biosearch	Custom
Probe: COVID-19_N2-P	IDT or Biosearch	Custom
Primer: RP-F	IDT or Biosearch	Custom
Primer: RP-R	IDT or Biosearch	Custom
Probe: RP-P	IDT or Biosearch	Custom
Template: 2019-nCoV_N_Positive Control	IDT or Biosearch	
Template: Hs_RPP30 Positive Control	IDT or Biosearch	

CONTROLS TO BE USED WITH THE COVID-19 RT-PCR

1. A "no template" control (NTC) serves as a negative control, and is included in every assay plate to identify specimen contamination. Molecular grade, nuclease free water is used as the NTC.

2. A positive template control is included in each assay plate to ensure the reagents and instruments are performing optimally. The positive control is a synthetic RNA (ultramers) containing the target sequence of gene N of the COVID-19 virus. Two markers in gene N, as defined by the "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel" document effective March 30, 2020, will be targeted and detected by the primer and probe sets, COVID-19_N1 and COVID-19_N2.

3. An internal control (Hs_RPP30 Positive Control) targeting human RNase P mRNA (RP) is used to verify optimal RNA extraction, amplification, and the presence of nucleic acid in the samples.

INTERPRETATION OF RESULTS

These controls will be analyzed on each plate.

- Positive control assays using ultramers for each N gene assay will be analyzed on each plate. Lung RNA will be used for the RNase P assay. These will be analyzed in the 30 Ct range to prevent issues due to template degradation.
- The extraction control with be the RNAse P assay.

Control results are interpreted as defined by the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel Instructions for Use.

Control Type	External Control Name	Used to Monitor	2019-nCoV_N1	2019-nCoV_N2	RP	Expected Ct Values
Positive	nCoV PC	Substantial reagent failure including primer and probe integrity	+	+	+	<40.00 Ct
Negative	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Extraction	HSC	Failure in lysis and extraction procedure, potential contamination during extraction	-	-	+	<40.00 Ct

The table below lists the expected results for the 2019-nCoV rRT-PCR Diagnostic Panel.

SARS-CoV-2 N1	SARS-CoV-2 N2	RP	Result Interpretation[a]	Report	Actions
+	+	±	SARS-CoV-2 detected	Positive SARS-CoV-2	Report results to state health department and provider.*
If only one of the two targets is positive and is ≤32		±	Inconclusive Result	Inconclusive	Repeat rRT-PCR if the repeated result remains inconclusive request another sample
If only one of the two targets is positive and is ≥32		±	Inconclusive Result	Inconclusive	Report as inconclusive and request another sample
-	-	+	2019-nCoV not detected	Not Detected	Report results to provider. Consider testing for other respiratory viruses.[b]
-	-	-	Invalid Result	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, request a new specimen from the patient.

[a] Diagnostic results will be reported as appropriate and in compliance with our specific reporting system

[b] Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus.

***** For at home collection from Everlywell, reporting will done through an Application Program Interfact to PWN.

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) -Analytical Sensitivity:

The LoD study was performed using viral genomic RNA from BEI using the CFX96. 10-fold serial dilutions of genomic RNA were spiked into pooled respiratory matrix (NP and OP swabs collected in liquid Amies) to obtain the LoD range. It was confirmed by 2-fold dilutions of RNA into matrix. The concentrations of RNA show the amount of RNA spiked into the matrix so the LoD was determined assuming 100% extraction efficiency.

Table 1. Limit of Detection Confirmation of the Assurance SARS-CoV-2 Panel with Abnova Total Nucleic Acid Purification Kit

Targets	2019-nCoV_N1		2019-nCoV_N2	
Concentration (genomic copies/µL)	9	5	9	5
Concentration (genomic copies/ reaction)	37	18	37	18
Positives/Total	20/20	20/20	20/20	20/20
Mean Ct	30.74	32.48	33.59	35.57
Standard Deviation (Ct)	0.29	0.36	1.01	0.45

Table 2. Limit of Detection Confirmation of the Assurance SARS-CoV-2 Panel with Zymo Research Quick-DNA/RNA Viral MagBead Kit

Targets	2019-nCoV_N1		2019-nCoV_N2	
Concentration (genomic copies/µL)	29	9	29	9
Concentration (genomic copies/reaction)	116	37	116	37
Positives/Total	20/20	20/20	20/20	20/20
Mean Ct	30.29	31.57	32.90	33.95
Standard Deviation (Ct)	0.33	0.35	0.96	0.61

The LoD was confirmed using the CFX384 to show that there is no loss in sensitivity with the 384 well plate (Table 3). The CFX384 had a lower LoD, but as both are used in the laboratory for routine testing the LoD was set according to the CFX96, 37 RNA copies/reaction.

Table 3. Limit of Detection Evaluation of the Assurance SARS-CoV-2 Panel with the CFX384.

	Zymo				Precipitor	
Targets	2019-nCoV_N1		2019-nCoV_N2		2019-nCoV_N1	2019-nCoV_N2
Concentration (genomic copies/µL)	29	9	29	9	29	29
Concentration (genomic copies/reaction)	116	37	116	37	116	116
Positives/Total	19/19	20/20	19/19	20/20	20/20	20/20
Mean Ct	26.86	27.84	29.19	29.95	26.40	27.78
Standard Deviation (Ct)	0.36	0.32	0.44	0.49	0.34	0.41

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2) Reactivity (Inclusivity):

An alignment was performed with the N1 and N2 oligonucleotide primer and probe sequences designed by the CDC with all publicly available nucleic acid sequences for 2019-nCoV in GenBank as of March 16th, 2019 to demonstrate the predicted inclusivity of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic panel. All the alignments show 100% identity of the CDC panel to the available 2019-nCoV sequences.

2019-nCoV-N1 Assay:
The reverse primer and probe sequences of the 2019-nCoV rRT-PCR assay N1 showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, the forward primer showed no sequence homology with Bat SARS-like coronavirus genome. Combining primers and probe, no significant homologies with human genome, other coronaviruses, or human microflora would predict potential false positive rRT-PCR results.

2019-nCoV-N2 Assay:
The forward and reverse primer sequence of 2019-nCoV rRT-PCR assay N2 showed high sequence homology to Bat SARS-like coronaviruses. The probe sequence showed no significant homology with human genome, other coronaviruses, or human microflora. Combining primers and probe, there is no prediction of potential false positive rRT-PCR results.

In summary, the 2019-nCoV rRT-PCR assay N1 and N2, designed for the specific detection of 2019-nCoV, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive rRT-PCR results.

3) Cross-reactivity (Analytical Specificity):

***In silico,* analysis has been performed and was reviewed by FDA (not shown because of large data set).**

In addition to the *in silico* analysis, nucleic acids were extracted from several organisms and tested with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic panel to demonstrate analytical specificity and exclusivity. Studies were performed with nucleic acids extracted using the Abnova Precipitor instrument using the Viral Total Nucleic Acid Purification Kit. Testing was performed using the ThermoFisher Scientific TaqPath 1-Step RT-qPCR Master Mix, CG on the BioRad CFX96 Real-Time PCR instrument. The data demonstrate the expected results are obtained for each organism when tested with the CDC N1 and N2 primers and probes.

Wet testing was performed with any organism that has greater than 80% homology to any primer or probe.

Wet testing results

Pathogens	Assays Evaluated		
	2019-nCoV	2019-	Final

Pathogens	Assays Evaluated		
	N1	nCoV N2	Result
Human coronavirus 229E	**0/3**	**0/3**	**Neg.**
Human coronavirus OC43	**0/3**	**0/3**	**Neg.**
Human coronavirus HKU1	**0/3**	**0/3**	**Neg.**
Human coronavirus NL63	**0/3**	**0/3**	**Neg.**
Adenovirus (e.g. C1 Ad. 71)	**0/3**	**0/3**	**Neg.**
Human Metapneumovirus (hMPV)	**0/3**	**0/3**	**Neg.**
Parainfluenza virus 1	**0/3**	**0/3**	**Neg.**
Parainfluenza virus 2	**0/3**	**0/3**	**Neg.**
Parainfluenza virus 3	**0/3**	**0/3**	**Neg.**
Parainfluenza virus 4	**0/3**	**0/3**	**Neg.**
Influenza A	**0/3**	**0/3**	**Neg.**
Influenza B	**0/3**	**0/3**	**Neg.**
Enterovirus (e.g. EV68)	**0/3**	**0/3**	**Neg.**
Respiratory syncytial virus	**0/3**	**0/3**	**Neg.**
Rhinovirus	**0/3**	**0/3**	**Neg.**
Chlamydia pneumoniae	**0/3**	**0/3**	**Neg.**
Haemophilus influenzae	**0/3**	**0/3**	**Neg.**
Legionella pneumophila	**0/3**	**0/3**	**Neg.**
Mycobacterium tuberculosis	**0/3**	**0/3**	**Neg.**
Streptococcus pneumoniae	**0/3**	**0/3**	**Neg.**
Streptococcus pyogenes	**0/3**	**0/3**	**Neg.**
Bordetella pertussis	**0/3**	**0/3**	**Neg.**
Mycoplasma pneumoniae	**0/3**	**0/3**	**Neg.**
Staphylococcus epidermidis	**0/3**	**0/3**	**Neg.**
Candida albicans	**0/3**	**0/3**	**Neg.**

4) Clinical Evaluation:

The experiments were performed using contrived samples generated by spiking viral genomic RNA into the pooled negative matrix (NP, OP and nasal swabs in liquid amies) from patients that were negative for SARS-CoV-2. For the non-reactive specimens, negative matrix was extracted without any additional spike.

For the Abnova Preciptor study 16 samples were prepared at LoD, 12 samples at 2xLoD and 10 samples were prepared across the range of the curve. Similarly, for the IndiMag 48, 24 samples were prepared at LoD and 11 samples were prepared across the range of the curve.

100% agreement was observed between the predicted results and actual results. All samples were run on the CFX96.

Contrived Samples Extracted with Abnova Precipitor

Assurance	Composite Comparator Result – Abnova Precipitor

SARS-CoV-2 Panel Result	N1		N2	
	Positive	Negative	Positive	Negative
Positive	**38**	**0**	**38**	**0**
Inconclusive	**0**	**0**	**0**	**0**
Negative	**0**	**30**	**0**	**30**

Positive percent agreement = 38/38 = 100%

Negative percent agreement = 30/30 = 100%

Contrived Samples Extracted with Zymo Research kit on the IndiMag

Assurance SARS-CoV-2 Panel Result	Composite Comparator Result – Zymo Research			
	N1		N2	
	Positive	Negative	Positive	Negative
Positive	**34**	**0**	**34**	**0**
Inconclusive	**0**	**0**	**0**	**0**
Negative	**0**	**48**	**0**	**48**

Positive percent agreement = 34/34 = 100%

Negative percent agreement = 48/48 = 100%

Clinical specimens received by Assurance Scientific Laboratories were tested by the Assurance Scientific Laboratories SARS-CoV-2 assay were confirmed by another clinical laboratory; Devansh Lab Werks Inc. using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. Results are below.

Assurance SARS-CoV-2 Panel Result	Reference result	
	Positive	Negative
Positive	5	0
Negative	0	5

Conclusion

Positive and negative percent agreement to expected result was 100% for the contrived swab specimens. Positive and Negative clinical specimens were also confirmed by secondary testing.